



A new species of *Rhinogobius* (Pisces: Gobiidae), with analyses of its DNA barcode

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Abstract

The genus *Rhinogobius* Gill 1859 is widely distributed in fresh waters along the Western Pacific coast of tropical and temperate Asia. A new species, *Rhinogobius maxillivirgatus*, is described from Anhui Province in Eastern China. This species can be differentiated from all congeners by a combination of the following characters: up to 6 longitudinal brown to black stripes along the side of the body; pectoral-fin rays modally 14; predorsal scale series 5–9; lateral scale series 28–30; transverse scale series 6–7; branchiostegal membrane with about 20 red round spots in males; and 2 black oblique stripes parallel along the upper jaw on the anterior portion of the cheek. Analyzing sequences of cytochrome c oxidase subunit I revealed that the new species is closely related to, but distinct, from *Rhinogobius wuyanlingensis*.

Key words: freshwater goby, *Rhinogobius maxillivirgatus*, Eastern China, *COI* gene

Introduction

Gobiidae is the second largest family among vertebrates in terms of species richness. The genus *Rhinogobius* Gill 1859 has the most species of freshwater gobies found within this family (Chen & Kottelat 2000; Nelson 2006; Nelson *et al.* 2016), which are widely distributed in fresh waters of coastal East Asia and islands of the Western Pacific. Species of *Rhinogobius* are very common in the lakes and streams of China, Russia, Japan, the Korean Peninsula, Vietnam, Thailand, Lao PDR, Cambodia, and the Philippines (Chen & Shao 1996; Sakai *et al.* 2000; Chen & Kottelat 2003, 2005; Wu *et al.* 2008; Akihito *et al.* 2013).

To date, over 80 species of *Rhinogobius* are recorded with nearly 50 species documented in China, most of which are endemic (Chen & Fang 2006; Li *et al.* 2007, 2009; Huang & Chen 2007; Chen *et al.* 2008; Wu *et al.* 2008; Chen & Miller 2008; Wu *et al.* 2009; Cao *et al.* 2016; Huang *et al.* 2016; Suzuki *et al.* 2017). Three migratory patterns are observed in this genus (amphidromous, fluvial, and lacustrine [Akihito *et al.* 2013; Tsunagawa *et al.* 2010]), but only non-diadromous species are found in South and East China (Huang & Chen 2007; Chen *et al.* 2008).

In 2016 and 2017, the authors (J.-H. X and Y.-Q. W.) surveyed the fish fauna in the mountains of southern Anhui province, China on three field trips (Fig. 1) and found a new species of *Rhinogobius* in the collected samples. The new species is described herein.

Materials and methods

Specimens were collected by hand-net. Those used for morphological comparisons were fixed in 10% formalin

immediately after capture and the ones for DNA barcoding were preserved in 99.7% ethanol. The taxonomic monograph *Fauna Sinica, Osteichthyes, Perciformes* (V), *Gobioidae* (Wu *et al.* 2008) was consulted for identification. Morphometric and meristic methods followed Akihito *et al.* (1984), Miller (1988), Chen & Shao (1996), Suzuki *et al.* (2017), and Yang *et al.* (2008). The measurements were taken with calipers to the nearest 0.1 mm. Body size was measured as standard length (SL). Terminology of the head lateral-line system followed Wongrat & Miller (1991). Counts of gill rakers include all rudiments on the outer side of the first arch. Vertebral counts (including urostyle) and dorsal pterygiophore formula (DP, the pattern of interdigitation of the dorsal-fin pterygiophores and neural spines) were obtained from a three-dimensional rendering scanned by X-ray microtomography (voltage 60kv, current 280µA, optimum 2.88 microns; NSI X50, U.S.A.) and reconstructed with the program efX-CT (North Star Imaging, Inc., U.S.A.). Meristic characters were abbreviated as follows: A, anal fin; C, caudal fin; D1, D2, first and second dorsal fins, respectively; LL, scales in lateral series from axilla along lateral midline; P, pectoral fin; PreD, predorsal scales; SDP, scale series from origin of first dorsal fin to upper pectoral-fin origin; TR, scales in transverse series from origin of A obliquely dorsally and posteriorly to base of second dorsal fin; and V, pelvic fin. Counts of gill rakers and head squamation were collected on a paratype (SNHM-F-2016040028).

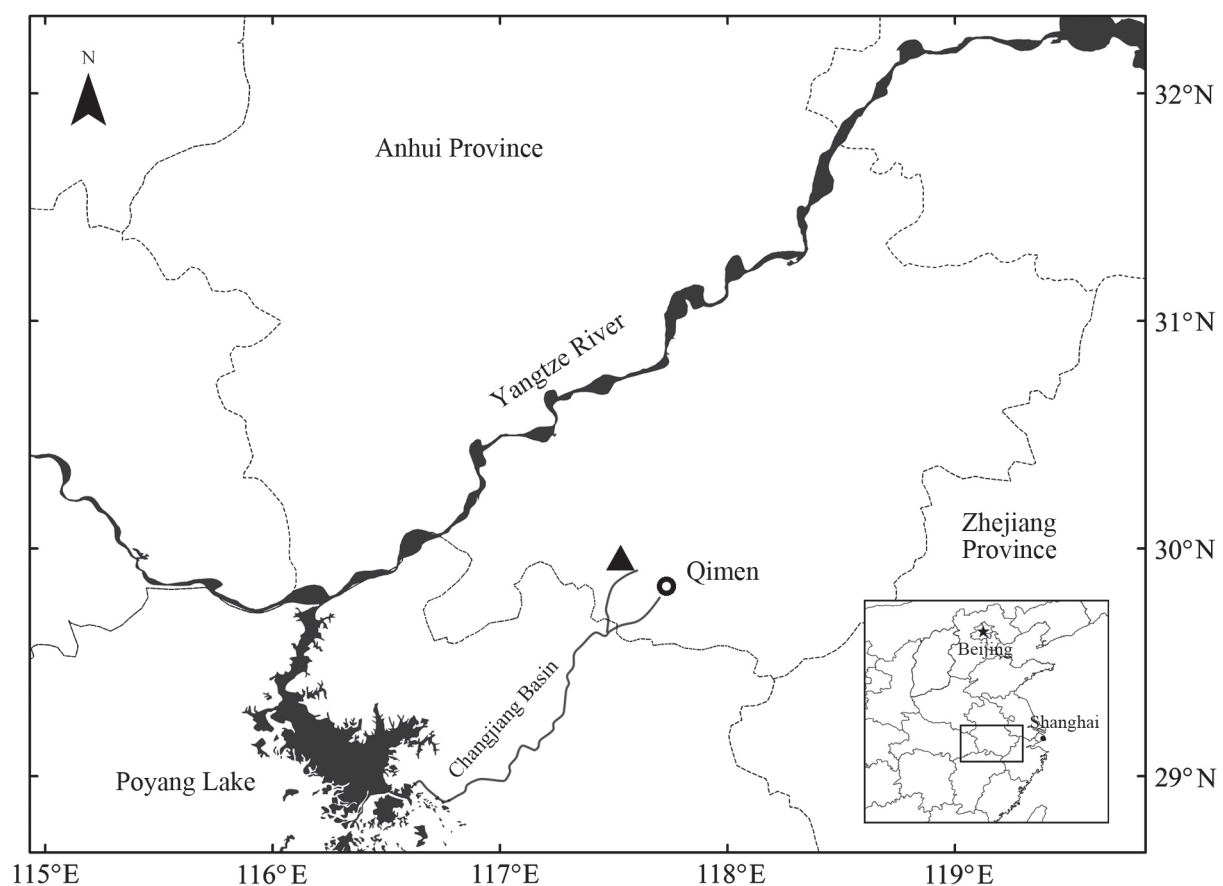


FIGURE 1. Sampling locality of *Rhinogobius maxillivirgatus* sp. n., marked with solid triangle.

The right pectoral fin was clipped from individuals preserved in ethanol for DNA barcoding. Types and specimens without the right pectoral fin were deposited in the Fish Collection of the Shanghai Natural History Museum (SNHM). Specimens of *Rhinogobius wuyanlingensis* Yang, Wu, & Chen that were used for comparison were deposited in the fish collection of the Shanghai Ocean University (SOU_CL1850_2 – SOU_CL1850_7).

Eleven specimens were used in the genetic analysis to evaluate the relationship of the new species to its congeners, including five individuals of the new species and six individuals of *R. wuyanlingensis*. In addition, *COI* sequences of *R. cliffordpopei* (NC_029252), *R. brunneus* (NC_028435), and *R. giurinus* (KU871066) were retrieved from GenBank for comparison. *Glossogobius olivaceus* (JQ001860) was used as an outgroup species.

Total genomic DNA was extracted from fin clips using an Ezup Column Animal Genomic DNA Purification Kit (Sangon, Shanghai, China). The whole length (~1500 bp) of the cytochrome c oxidase subunit I (*COI*) gene was amplified with custom primers *Rhinogobius_COI_L* (GGCTACAATCCACCACTTAAGTCTCA) and *Rhinogobius_COI_R* (TTCRACTCCTCCCTTTCTCGTT). The PCR was carried out in total reaction volumes of 25 µL, on a cycling profile of 95°C for 3 min, 16 cycles of 95°C for 50 sec, 57°C for 1 min, and 72°C for 1 min, followed by 16 cycles of 95°C for 50 sec, 55°C for 1 min, and 72°C for 1 min, ending with 72°C for 10 min. The PCR products were sequenced from both ends by Shanghai Sangon Bioscience and Technology Incorporation, Shanghai, China. Resulting sequences were assembled and manually checked using CodonCode aligner 6.0.2 (Centerville, MA, USA). The validated sequences were deposited in GenBank (MG272362 – MG272372). Sequence alignment and calculation of pairwise p-distance were performed using MEGA v7.0.26 (Kumar *et al.* 2016). The best nucleotide substitution model was chosen based on BIC criterion using model selection implemented in MEGA v7.0.26. A Maximum Likelihood (ML) phylogeny with 1000 bootstraps was reconstructed under the best model using MEGA v7.0.26.

Results

Rhinogobius maxillivirgatus Xia, Wu, and Li, sp. n.

(Figure 2)

Holotype. SNHM-F-2017060018, female, 30.1 mm SL; a tributary of Changjiang River, Qimen County, Huangshan City, Anhui Province, China; 4 June 2017, J.-H. Xia.

Paratypes. SNHM-F-2016040025, male, 30.8 mm SL; SNHM-F-2016040026, male, 28.0 mm SL, 28 April 2016; SNHM-F-2016100025, male, 23.0 mm SL; SNHM-F-2016100090, male, 24.3 mm SL, 12 October 2016; SNHM-F-2016040027, female, 31.1 mm SL; SNHM-F-2016040028, female, 29.3 mm SL; SNHM-F-2016040029, female, 31.1 mm SL, 28 April 2016; SNHM-F-2016100032, female, 25.1 mm SL; SNHM-F-2016100091, female, 23.8 mm SL, 12 October 2016. Locality same as holotype, J.-H. Xia & Y.-Q. Wu.

Other material. Five additional specimens were used for DNA barcoding, not measured: 4 females (SNHM-F-2016100002, SNHM-F-2016100005, SNHM-F-2016100093, SNHM-F-2016100095) and 1 male (SNHM-F-2016100094); 12 October 2016, locality same as holotype, J.-H. Xia & Y.-Q. Wu.

Diagnosis. *Rhinogobius maxillivirgatus* is distinguishable from the other species in the same genus by the unique combination of the following features: D2 rays modally I/8 (rarely I/9); A rays modally I/7; P rays 13–15; LL 28–30; TR 6–7; predorsal scales 5–9; vertebral count 10+17=27; lateral side with 5–6 longitudinal serrated stripes, ventral ones dark; a whitish band across from snout to dorsal portion of operculum, interrupted by orbit; a black stripe along lower half of upper jaw, followed by a longer parallel stripe across anterior portion of cheek; rear edge of orbit with a black blotch; cheek and operculum spotless, branchiostegal membrane grayish with up to 20 red spots in male; D1 with 3–4 horizontal rows of spots and D2 with 5–6 rows; P base with an arched reddish brown band, membrane with several vertical rows of spots and a bold mark near the origin; and C base with a large dark spot, with vertical rows of spots in membrane and the anterior row enlarged.

Description. Morphometric and meristic data for the holotype and paratypes are presented in Table 1. Body slim, rather cylindrical anteriorly, compressed posteriorly. Eyes dorsolateral, diameter larger than interorbital width. Head moderately conical; snout small, cheeks fleshy. Lips thick. Mouth oblique; lower jaw slightly longer than upper, both with 4–5 rows of conical teeth, outer row enlarged. Isthmus broad, united to branchiostegal membrane. Gill rakers 3+7. Genital papilla flat and triangular in males; column-like with terminal pore in females.

Fins. D1 rays VI (rarely V) with longer third and fourth spinous rays, never extending to base of first branched ray of D2 when adpressed in both sexes. D2 rays modally I/8; A rays modally I/7; P rays 13–15; V rays I/5+I/5. Rear tips of D2 and A rays not reaching procurent rays of C when adpressed, A origin inserted below second branched ray of D2. V resembling disc, with spinous rays with pointed membranous lobe. P and C elliptical, distal margin of C rounded.

Scales. Body with ctenoid scales, mid-trunk scales enlarged. Breast, prepelvic area, and almost whole head naked. PreD cycloid, 5–9; LL 28–30, TR 6–7, SDP 5–7. Anterior part of predorsal area naked. Predorsal squamation with trifurcate anterior edge and anterior boundary of middle series extending just above canal pore θ , boundary of anterior extension on side of occipital region reaching dorsal origin of pectoral fin.

TABLE 1. Morphological and meristic data for *Rhinogobius maxillivirgatus* from Anhui Province, China, n=10. Ranges of female specimens include holotype. The catalog numbers of type specimens are listed in results.

Variable	Holotype	Min–Max (Mean)	
Sex	Female	Male	Female
Number	1	4	6
Standard length (mm)	30.1	23.0–30.8 (26.5)	23.8–31.1 (28.4)
Head length (mm)	8.6	7.1–9.2 (8.1)	6.6–8.6 (7.8)
Percent standard length (%)			
Head length	28.6	29.9–30.9 (30.4)	25.7–28.6 (27.4)
Predorsal length	39.8	38.3–41.1 (39.7)	37.0–40.8 (38.5)
Snout to second dorsal-fin origin	58.8	57.0–59.6 (58.8)	57.0–59.7 (58.4)
Snout to anal-fin origin	63.5	59.1–61.8 (60.4)	59.8–63.5 (61.2)
Snout to anus	57.8	55.1–57.1 (55.7)	52.4–57.8 (54.6)
Prepelvic length	26.2	30.9–32.1 (31.7)	26.2–32.8 (30.2)
Caudal peduncle length	25.2	25.2–28.6 (27.1)	24.8–27.3 (26.4)
Caudal peduncle depth	10.6	9.1–10.4 (9.6)	8.4–10.6 (9.6)
First dorsal-fin base	15.6	12.8–17.9 (14.7)	11.6–16.4 (14.0)
Second dorsal-fin base	19.9	16.9–18.5 (17.6)	15.9–19.9 (17.2)
Anal-fin base	16.3	14.4–16.5 (15.4)	13.0–17.0 (14.7)
Caudal-fin length	21.9	23.0–24.8 (24.0)	21.9–24.4 (23.2)
Pectoral-fin length	22.9	19.3–24.0 (21.3)	21.4–24.7 (22.7)
Pelvic-fin length	14.3	13.6–16.5 (14.7)	12.2–16.0 (14.6)
Body depth of pelvic-fin origin	16.3	10.0–14.6 (13.0)	12.4–16.3 (14.5)
Body depth of anal-fin origin	13.0	11.9–13.2 (12.5)	12.6–14.3 (13.2)
Body width of anal-fin origin	10.0	7.4–9.7 (8.6)	7.2–11.3 (9.6)
Pelvic-fin origin to anus	31.2	21.8–25.2 (23.8)	23.5–31.2 (25.9)
Percent head length (%)			
Snout length	24.4	21.7–25.9 (24.0)	23.8–26.5 (25.1)
Eye diameter	23.3	21.7–26.8 (23.8)	22.6–27.3 (24.2)
Cheek depth	9.3	5.4–10.9 (7.9)	6.0–9.3 (7.3)
Postorbital length	51.2	47.3–53.5 (50.9)	47.1–51.2 (49.4)
Head width in maximum	69.8	47.9–60.0 (54.8)	59.1–69.8 (63.8)
Bony interorbital width	4.7	5.4–8.7 (7.1)	4.7–7.6 (6.3)
Lower jaw length	20.9	26.8–31.5 (29.7)	20.9–30.3 (24.8)
Meristic count		Mode (Min–Max)	Frequency (%)
D1	VI	VI (V–VI)	90
D2	I,9	I,8 (I,8–I,9)	90
A	I,8	I,7 (I,7–I,8)	80
P	15	15 (13–15)	50
LL	30	28 (28–30)	40
TR	7	7 (6–7)	60
SPD	6	6 (5–7)	60
PreD	7	7 (5–9)	40

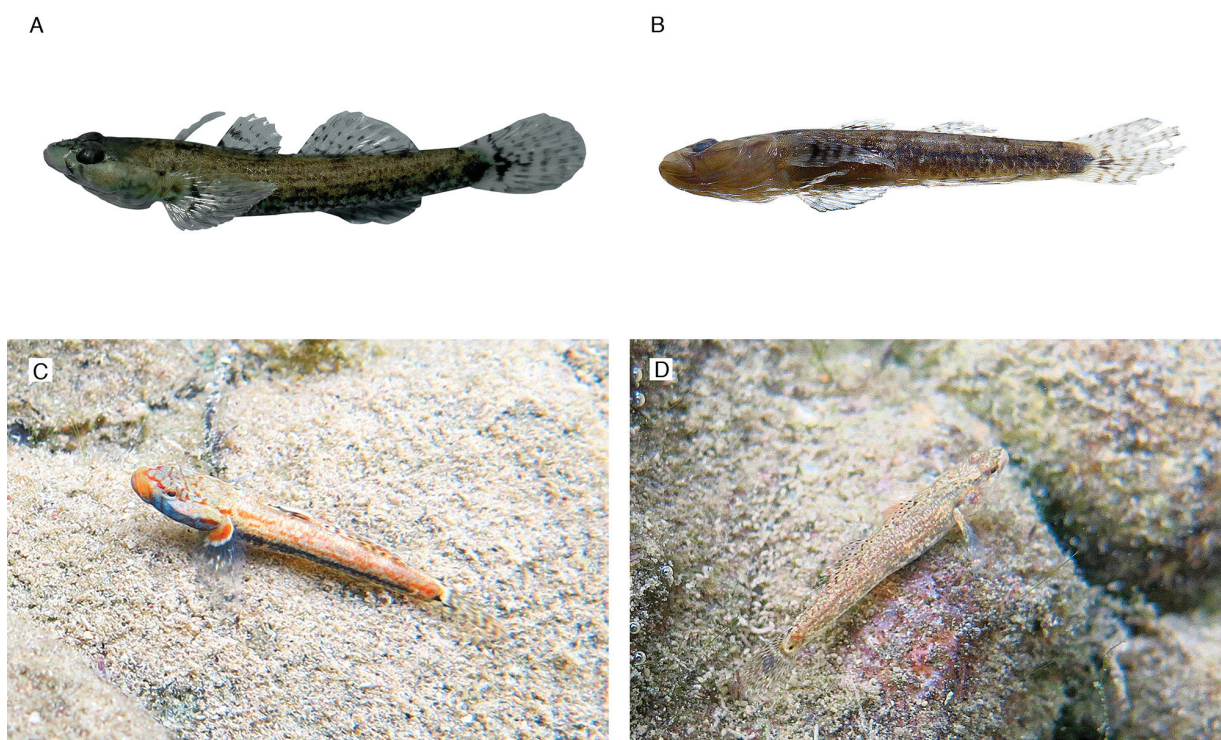


FIGURE 2. *Rhinogobius maxillivirgatus* **A.** Holotype, SNHM-F-2017060018, Female, 30.1 mm SL, a tributary of upper reach of Changjiang River, Qimen County, Anhui Province, China; **B.** Paratype, SNHM-F-2016040025, Male, 30.8 mm SL; **C.** Male adult at type locality; **D.** Female adult at type locality.

Cephalic canal pores: See Fig. 3 A–B. Tubular anterior nares and posterior nares, simple pores without any rim. Canal pore arrangement complete for the genus. Nasal extension of anterior oculoscapular canal with paired terminal pore σ , located anterior to posterior nares, but not reaching anterior nares; anterior interorbital sections separated, with paired pore λ and single pore κ ; lateral section over cheek with pore α and terminal pore ρ ; pore ω present near posterior border of eye between α and κ . Posterior oculoscapular canal quite short, with two terminal pores θ and τ . Gap between pores ρ and θ greater than length of posterior oculoscapular canal. Pores γ , δ , and ϵ along preopercular canal, distance between γ and δ shorter than that between δ and ϵ .

Sensory papillae. See Fig. 3 A–C. Row *a* short, extending ventrally along rear edge of orbit, ventral terminal not reaching vertical midline of orbit. Row *b* extending below orbit, longer than *a*, but not reaching posteriorly to vertical line through rear margin of orbit. Row *c* stretching longitudinally on middle cheek, with nearly half length of row *b*. Row *d* oblique, along lower half of upper jaw. Operculum with rows *ot*, *oi*, and *os*; row *oi* attaching ventrally to ventral part of row *ot*; row *os* extending ventrally towards posterior, well separated from rows *ot* and *oi*. Preopercular-mandibular papillae with rows *e*, *i*, and *f*, paired respectively.

Vertebrae. Shown in Fig. 4A. Total vertebrae $10 + 17 = 27$. DP 3/II II I I 0/9

Coloration. See Figs. 2 and 4 B–C. Body always with 5–6 longitudinal serrated stripes from dorsum to venter; stripes long and parallel, ventral ones darker. Belly pale. Whitish band running from upper jaw to posterior operculum, interrupted by orbit. Dorsum of snout with U-shaped line united at tip of snout. Black blotch behind orbit. Oblique stripe along lower half of upper jaw, followed by parallel black stripe originating from lower anterior edge of orbit; longer than the previous. Males with red lips in breeding season, faded in fixed material. Branchiostegal membrane pale yellow with up to 20 red spots in males after fresh preservation; spotless in females. Dorsal midline covered with 5 large black blotches.

Fins with rows of black spots alternating with bright rows of white spots in living specimens; whitish rows disappearing when preserved. D1 with 3 longitudinal rows of black spots; D2 with 5 rows. C with 6–7 vertical waved rows of black spots and middle portion of anterior row with a large blotch; base with a large black spot in center, attached to shiny transverse band in life, fading in preservation. P base with deep red mark, followed by bright arched band in live males, red mark vanishing in preservation; 5–6 vertical rows of black spots and anterior row enlarged as a band in both sexes. V grayish in male, whitish and uniform in female.

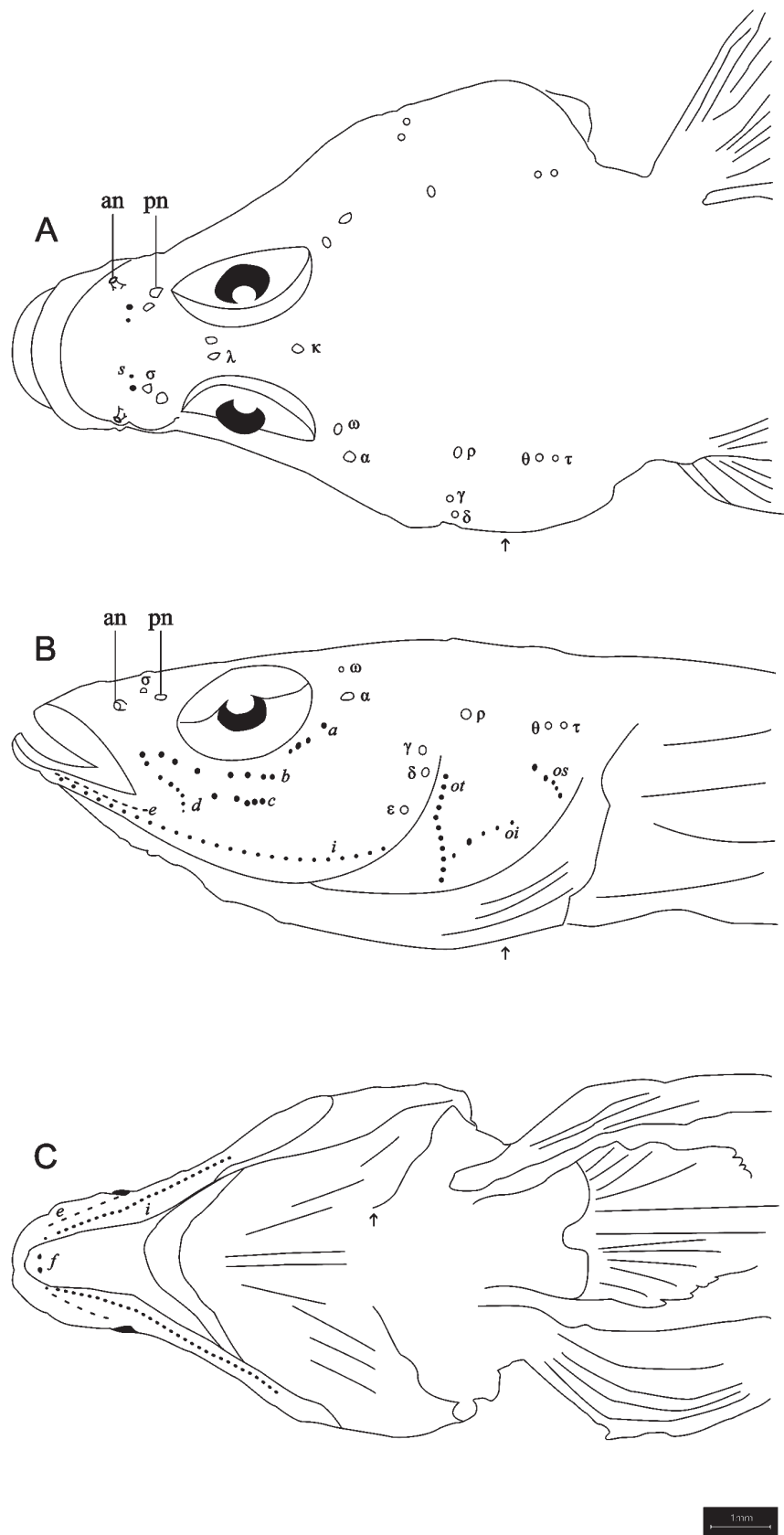


FIGURE 3. Head lateral-line system of holotype (SNHM-F-2017060018, female, 30.1 mm SL) of *Rhinogobius maxillivirgatus* sp. n. in **A.** dorsal, **B.** lateral, and **C.** ventral views. Arrow shows ventral edge of gill slit. an, pn, anterior and posterior nares; other characters indicate cephalic canal pores and sensory papillae.

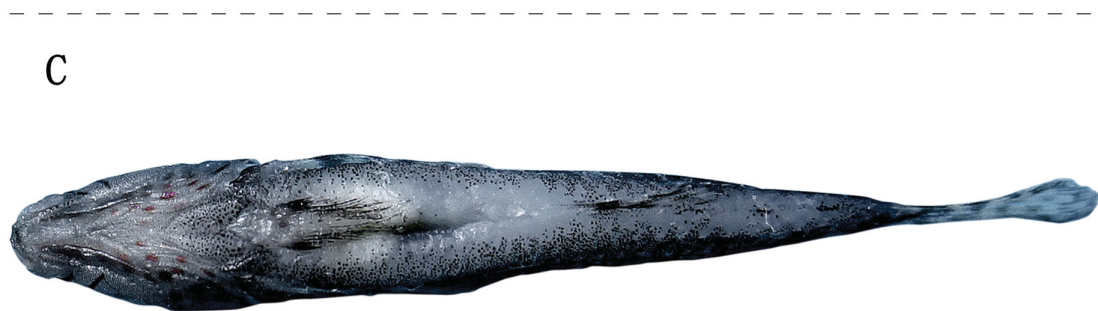
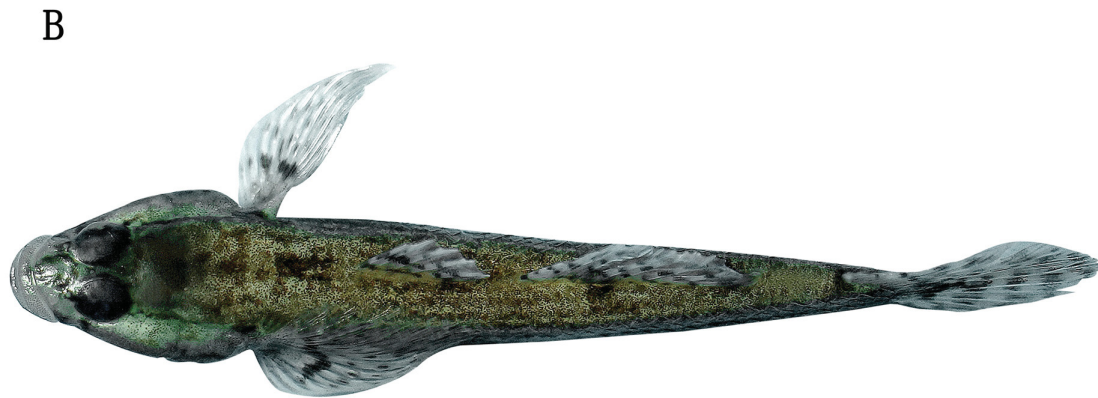
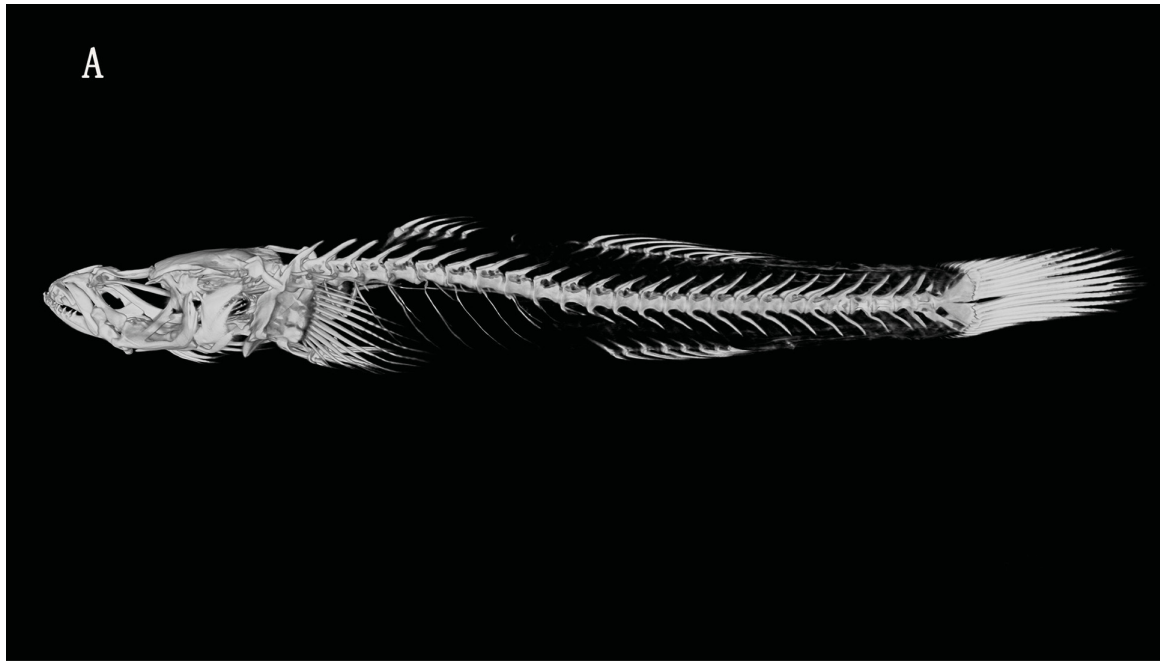


FIGURE 4. *Rhinogobius maxillivirgatus* sp. n. **A.** Three-dimensional rendering of a paratype based on X-ray microtomography in lateral view, SNHM-F-2016040026, male, 28.0 mm SL; **B.** Five blotches along dorsal midline of holotype, SNHM-F-2017060018, female, 30.1 mm SL; **C.** Branchiostegal membrane with up to 20 red spots in fresh preservation of a paratype, SNHM-F-2016100025, male, 23.0 mm SL.

Distribution. Currently *Rhinogobius maxillivirgatus* is only known from a particular tributary of the Changjiang Basin in Qimen County, Huangshan City, Anhui Province, China.

Etymology. The name *maxillivirgatus* is a noun in apposition and derived from the Latin *maxilla* meaning “upper jaw” and *virgas* meaning “stripes,” referring to 2 distinctive stripes behind the upper jaw of both sexes.

Biology. This species inhabits a shallow, slow-flowing brook of the upper Changjiang River, with a width of 5–6 meters and cobble substrate. According to three field trips, it was observed that breeding happens from late April to middle June and the number of gravid individuals increased in early June.

Phylogenetic analysis. The best model selected was HKY + G. *Rhinogobius maxillivirgatus* is a sister species to *R. wuyanlingensis* in the ML tree rooted with *G. olivaceus* (Fig. 5). Average genetic p-distance between *R. maxillivirgatus* and *R. wuyanlingensis* is 0.081, which is comparable to the genetic distance between *R. cliffordpopei* and *R. brunneus* (0.097).

Remarks. *Rhinogobius maxillivirgatus* is most similar to *R. wuyanlingensis*, from neighboring Zhejiang Province in Eastern China. Both species have a slim body with about 6 longitudinal lines from the dorsal to ventral region (Yang *et al.* 2008). However, *R. maxillivirgatus* can be distinguished from *R. wuyanlingensis* by possession of 1) P rays modally 14 (vs. 17–18, modally 18); 2) LL 25–30 (vs. 30–32); 3) TR 5–7 (vs. 9–10); 4) 2 oblique stripes along upper jaws on anterior area of cheek (vs. lacking such pattern of stripes); and 5) branchiostegal membrane with about 20 red big spots in male (vs. 6–7 transverse deep red stripes on branchiostegal membrane in males) (features in parentheses, from Yang *et al.* [2008]).

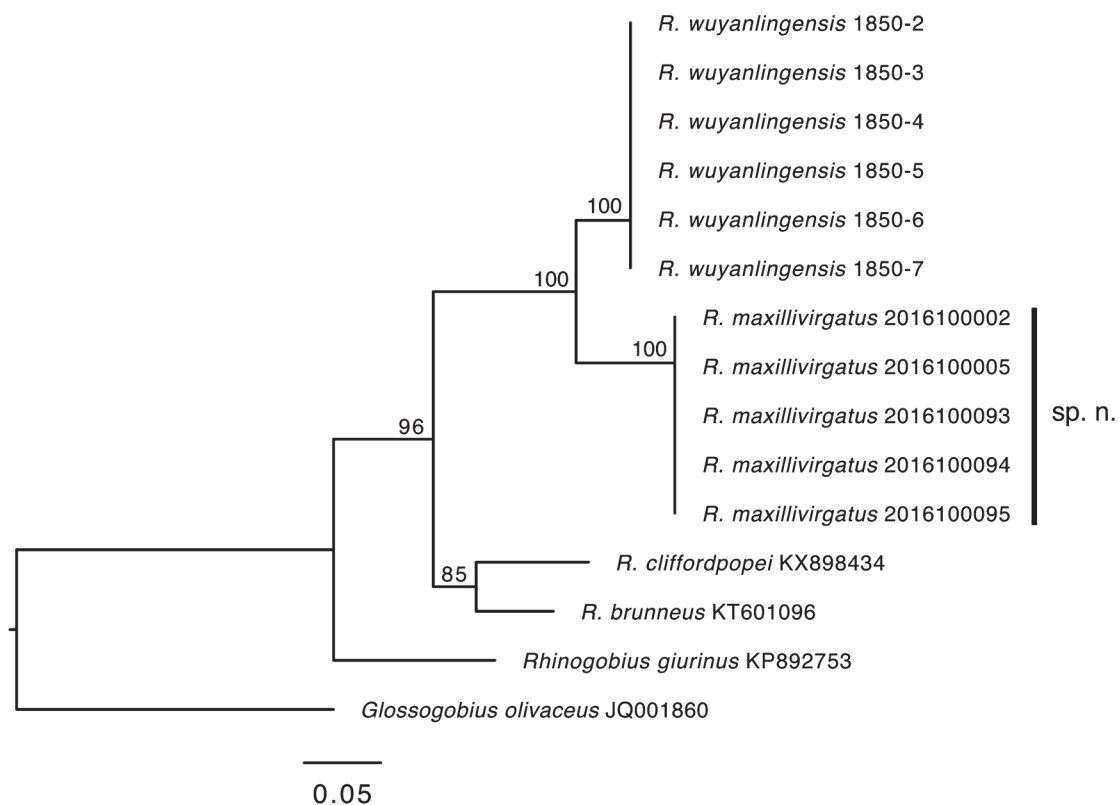


FIGURE 5. Maximum likelihood tree of *Rhinogobius maxillivirgatus* sp. n., with congeners based on sequences of full length of cytochrome c oxidase subunit I. The values at nodes are bootstrap support values.

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